

ABSTRACT

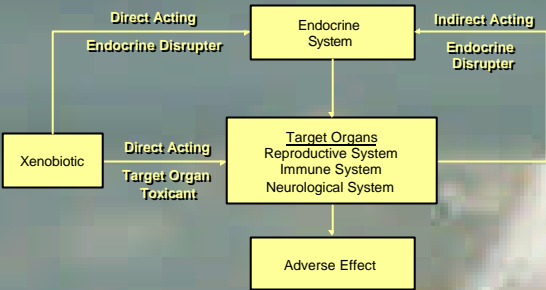
Grass shrimp are an ecologically important species in estuarine ecosystems, effectively transforming nutrients into a bioavailable form. Additionally, they serve as a major source of food to other species. Decreases in grass shrimp populations may be an indicator of environmental contamination. Several ecosystems with significant contamination (PAHs, pesticides, and metals) have been found to have reduced grass shrimp populations (Murrells Inlet, SC; Shipyard Creek, SC; Koppers Creek, SC) when compared to a reference site. Our previous work has shown that reproduction in grass shrimp is decreased by chronic endosulfan exposure (200 ng/L) but is not affected by methoprene (1 mg/L). Pooled shrimp from each treatment were extracted and analyzed for total lipid, lipid class composition, and fatty acid composition analysis in control and treated populations. Results indicated that total lipids were significantly reduced in both endosulfan and methoprene treatments when compared to controls. Triacylglycerol levels (in terms of the percent of total lipids) in the methoprene treated shrimp were significantly increased compared to controls. Shrimp exposed to this insecticide had a non-significant reduction in the relative amount of sterols, but absolute amounts for each lipid class were significantly reduced (except for the triacylglycerols) when compared to controls. Two fatty acids (18:3n-3 and 22:6n-3) were found to be significantly altered in the methoprene exposed shrimp. The reduced reproduction that has been observed in grass shrimp following endosulfan exposure may be due in part to an alteration in the absolute amounts of either the main nutritional lipid compartment (triacylglycerol) or sterols. This reduction in the energy stores derived from the triacylglycerols could effectively reduce the ability of shrimp to become gravid by diverting energy reserves to growth and survival rather than reproduction.

OBJECTIVE

Evaluate the effects of insecticide (endosulfan and methoprene) exposure on the lipid composition and carbon:nitrogen ratio of the grass shrimp, *Palaemonetes pugio*.

BACKGROUND

The US EPA model (Kavlock et al., 1996) for xenobiotics which act as endocrine disrupting chemicals may include alterations in lipid synthesis or uptake. Any alteration in normal lipid homeostasis via exposure could indirectly affect endocrine functions and/or reproduction.



The nutritional state of an organism plays an important role in its ability to maintain normal physiological and reproductive functions. Alterations in lipid-associated endpoints may be indicative of future reproductive impacts within a population. To assess the potential for chronic pesticide exposure to affect normal lipid accumulation (via synthesis and dietary uptake) reproductively active females were sampled for several lipid and protein endpoints. The major lipid classes in the grass shrimp are phospholipids (PL, structural components), sterols (STER, hormone synthesis and structural) and triacylglycerols (TAG, energy storage).



# LIPID ALTERATIONS IN GRASS SHRIMP (*PALAEMONETES PUGIO*) EXPOSED TO METHOPRENE AND ENDOSULFAN

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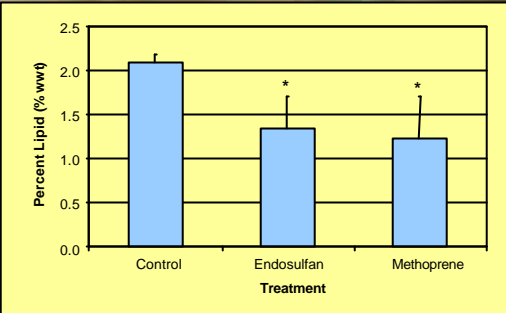
METHODS

- Barren females (60) and males (20) were exposed to endosulfan (20% of the male LC50; 200 ng/L nominal), methoprene (1 mg/L nominal) or controls in 64L aquariums for up to 35 days and allowed to mate.
- Gravid females were removed and frozen daily, a subset of these tissues were analyzed for lipid endpoints, protein content and carbon:nitrogen (C:N) ratios.
- Lipids were extracted from three replicates of shrimp from control, endosulfan, and methoprene treated populations using the Folch (1952) method and reduced to 5 mL final volume.
- Total lipids were measured gravimetrically, averaging two 0.5 mL aliquots of the reduced Folch extract.
- The relative percent lipid class for the three major classes (PL, STER, TAG) were determined using thin layer chromatography with flame ionization detection (TLC-FID, latroscan Mark IV).
- Fatty Acid (FA) profiles for PL and TAG from exposed and control treatments were derived and compared. PL and TAG fractions were collected from silica SPE cartridges, converted to fatty acid methyl esters (BF<sub>3</sub> in 14% methanol), analyzed by GC-FID, and quantified as wt % of fatty acids. FA were identified by comparison of peak retention times with those of a known standard and by GC-MS.
- Total protein concentration in both females was quantified using the Lowry method (Lowry et al, 1951).
- C:N ratio analysis using the Dumas method was performed by Dr. Bob Petty, Analytical Laboratory, Marine Science Institute, UC-Santa Barbara on dried and powdered tissues
- Statistical comparisons of the two insecticide treatments were compared to controls using a one-way ANOVA and Dunnett's procedure, p=0.05.

RESULTS

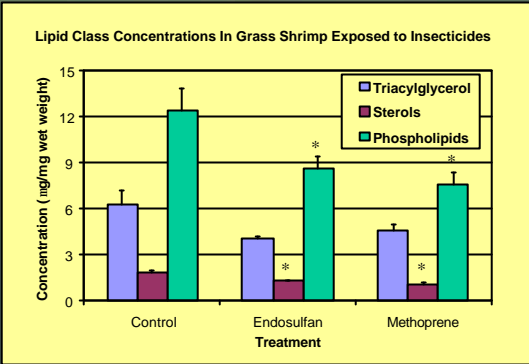
Total Lipids

The average (+/- std. dev.) percent total lipid measured in *P. pugio* on a wet weight basis showed a significant decrease when each treatment was compared to controls (\*, p<0.05).



Lipid Class Composition

Graphically, the decrease in total lipids in the endosulfan treatment does not alter the general profile of the lipid classes. Methoprene treated shrimp appear to alter the relative amounts of the lipid classes, effectively altering the lipid class profiles. In both treatments, the absolute amounts of each class (with the exception of the TAG) was significantly reduced compared to controls.



Major lipid classes reported as a percent of total lipids, were quantified from female grass shrimp and compared to controls (significantly different from controls (p < 0.05) is indicated by **bold**). The levels of triacylglycerols found in the methoprene treated females were significantly different from the controls and sterol levels were non-significantly reduced by ~10%.

Lipid Class (% of Total Lipid)	TREATMENT		
	Control	Endosulfan	Methoprene
<b>Phospholipids</b>			
Average	60.4	61.3	57.8
Standard Deviation	1.6	2.8	2.2
<b>Sterols</b>			
Average	9.2	9.4	7.5
Standard Deviation	1.8	0.6	1.6
<b>Triacylglycerols</b>			
Average	30.4	29.3	<b>34.7</b>
Standard Deviation	1.8	2.2	2.3.

Analysis of fatty acids indicated alterations of two individual FA in both the PL and TAG fractions. A significant decrease in the FA 22:6n-3 was seen in the methoprene treated PL and TAG fractions while a significant increase in the levels of 18:3n-3 were seen. A similar trend but statistically non-significant change was seen in the endosulfan treated shrimp.

	Triacylglycerol Fraction				Phospholipid Fraction			
	Average	Std.	Error		Average	Std.	Error	
	(wt-% total)	(wt-% total)			(wt-% total)	(wt-% total)		
	18:3n-3	22:6n-3			18:3n-3	22:6n-3		
Control	4.50	0.781	4.20	0.257	2.90	0.572	7.77	0.136
Endosulfan	6.37	0.781	3.27	0.257	4.10	0.572	7.47	0.136
Methoprene	<b>8.37</b>	0.781	<b>3.03</b>	0.257	<b>5.13</b>	0.572	<b>6.60</b>	0.136

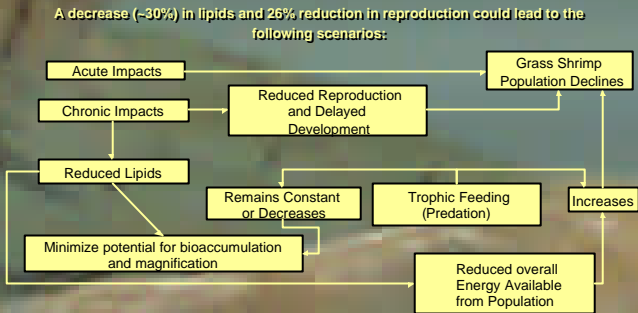
Protein concentrations from adult females quantified using a modified Lowery method showed no differences in treated individuals when compared to the controls. Additionally, female grass shrimp were analyzed for C:N ratios.

	Average Protein (mg/g ww)	Std. Dev.	P-value	Average C:N Ratio	Standard Deviation
Control	146.5	39.43	N/A	3.903	0.274
Endosulfan	162.6	49.25	0.68	3.903	0.161
Methoprene	175.2	49.74	0.48	4.067	0.180

CONCLUSIONS

- Exposure to endosulfan and methopene reduced total lipid content by 33-37%
- Methoprene treated shrimp exhibited a significant increase in the percent of total lipids which were accounted for by the TAG fraction.
- Absolute tissue concentrations were significantly reduced for STER and PL in both insecticide treatments. Amounts of TAG were reduced non-significantly in both treatments.
- There were no differences in protein concentrations between treated and control shrimp
- There were no significant differences in C:N ratios

Potential Impacts of Reduced Lipid and Reproduction: *Ecological Importance*



SELECTED LITERATURE

Folch, J., Lees, M. and Sloane Stanely, G.H. 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226:497-509.

Lowry, O., Rosebrough, N., Farr, F. and Randall, R. 1951. Protein measurements with the folin phenol reagent. J. Biol. Chem. 193:265-275.

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